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NEW APPROACHES TO PREVENTION OF NSAID-GASTROPATHY

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Abstract

Introduction: At present, the issue of gastric mucosal damage, induced by the use of non-steroidal anti-inflammatory drugs, remains unresolved.

Objectives: The development of new methods of prevention of NSAID-gastropathies with oral coursework use of taurine and procaine, as well as the study of cellular mechanisms of the damaging effect of diclofenac sodium and Ketorolac tromethamine.

Methods: The methodological approach was based on a range of theoretical, pharmacological, histological, statistical, biophysical methods.

Results and discussion: Diclofenac sodium and ketorolac tromethamine, being in direct contact with cell membranes, cause a change in the structural and functional properties that present in the defect formation. This resulted in a decrease in the acid and hypo-osmotic resistance of model cells due to the broken or weakened bonds stabilizing the proteins molecules in membrane (which is associated with the dissociation of NH+-groups of the imidazole ring of histidine, the terminal α-amino groups (not less than 10.5% relative to the control), sulfhydryl groups of cysteine, phenolic groups of tyrosine, ε-amino groups of lysine (not less than 8.7%)). In experiments in vitro and in vivo procaine reduces the damaging effect of Ketorolac trometamina 28% and 19.7%, respectively, the formation of hidden defects reduced by 69% when taurine cellular damage was reduced by 54% and 19.7% of the latent defects is less than 74%.

Conclusion: Prophylactic intragastric administration of procaine or taurine for 7 days before ketorolac tromethamine administration significantly reduces the amount of erosive and ulcerative defects (87% and 90%, respectively).

Keywords: Procaine, taurine, nonsteroidal anti-inflammatory drugs, membrane damage, prophylaxis, NSAID-gastropathy.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in medical practice [1, 2, 3]. However, the issue of tolerability and safety of NSAIDs is particularly urgent [1, 4]. Non-steroidal anti-inflammatory drug gastropathy (NSAID-gastropathy) is a specific syndrome, mainly presented by the gastric mucosal lesions with the development of erythema, erosions and/or ulcers, and is recognized as one of the most common serious complications of NSAID therapy. Currently, research is being conducted to identify unknown mechanisms of NSAID-gastropathy and to develop new drugs for the treatment and prevention of complications [5, 6].

To prevent and treat NSAID-dependent gastropathies, proton pump inhibitors, histamine H2-receptor antagonists, antacids, mechanical protectors for the erosive-ulcerative lesion, as well as new drugs, e.g. rebamipide

gastroprotector – synthetic analogue of prostaglandin E2 – are used [7, 8]. However, histamine H2-receptor inhibitors, local bismuth preparations and antacids have been established to be ineffective for the treatment and prevention of gastric ulcers in patients taking non-steroidal anti-inflammatory drugs for a long time [9, 10].

Of all these drugs, proton pump inhibitors are considered to be the most effective. However, their efficiency is reduced by localization of lesions in the gastric mucosa, prolonged use of NSAIDs and the absence of H. pylori [11]. In addition, long-term use of drugs reducing the acidity of gastric juice (histamine H2-receptor blockers, proton pump inhibitors, antacids) increases intragastric pH and is capable of causing digestion disorders, which is manifested by the clinical picture of dyspeptic syndrome. On the one hand, a prolonged increase in pH significantly weakens the barrier to pathogenic and potentially pathogenic flora entering the gastrointestinal tract. On the other hand, the persistent suppression of gastric acid secretion causes hypergastrinemia which is fraught with development of dis- and metaplastic processes in the gastric epithelium (against a background of chronic inflammation) [5, 12]. Therefore, the development of new ways of preventing NSAID-gastropathy and clarifying the mechanisms of the damaging effect of NSAIDs at the cellular level are topical issues [13, 14].

Materials and methods

The drugs used in the study are Diclofenac (Lotus Laboratories Pvt. Ltd., India), Ketorol (Dr. Reddy's Laboratories Ltd., India), procaine substances (No. LS-000007, 2010-01- 18 HubeiMaxpharmIndustriesCo, China) and taurine (FS.2.1.0039.15; CAS: 107-35-7, CJSC "Vekton", Russia). Experimental studies were conducted on 503 white mongrel male rats with an initial weight of 200-250 g and 225 white mongrel mice of both sexes weighing 20-25 g, obtained from the vivarium of the Voronezh N.N. Burdenko State Medical University. Animals were kept in vivarium conditions, T = 17-24 °C, under natural light conditions, 50-70% humidity, and were fed with conventional combined fodder [15].

Study of the ulcerogenic effect of ketorolac tromethamine

The experiments were carried out in accordance with the recommendations of the "Guidelines for experimental (preclinical) study of new pharmacological substances" [16], using the "Biomed-1" microscope (research and production company Delta Trans LLC, Russia). The design of the study is shown in the figure (Fig. 1)

Study of the blood coagulation system

Coagulograms were recorded on the automated coagulation analyzer (model "H 334", JSC "Krasnodar ZIP", Russia) [17], the design and doses of the experiment being similar to the design of the ulcerogenic experiments.

Study of the structural and functional properties of erythrocyte membranes. Spectrophotometric method was used ("PE 5400 VI" spectrophotometer, "Ekohim" Ltd., Russia) for the registration of hypo-osmotic acid resistance of erythrocytes placed in the hypo-osmotic solution of sodium chloride (0.55%) [18], or by adding of 0,1M hydrochloric acid solution to 5 ml of erythrocyte suspension [19]. The main analyzed erythrogram indices calculated by the equation [18] were as follows: 1) K_{max} – constant maximum speed of erythrocyte hemolysis (relative units), G_{sph} – relative amount of spherocytes (%), G_{120} – hemolyzed erythrocytes in the hypo-osmotic environment for 120 seconds (%). The blood of 230 white mongrel rats was used. The design of the experiments is shown in the figure (Fig.1).

Study of the optical properties of proteins

Spectrophotometric method was used ("PE 5400 VI" spectrophotometer, "Ekohim" Ltd., Russia) [20], oxyhemoglobin solutions were prepared of the blood of 95 white mongrel male rats, the concentration was monitored spectrophotometrically (D = 0.8). The design of the study is shown in the figure (Fig. 1).

Study of alkaline buffer capacity of proteins with acid-base titration. The degree of modifier effect to physicochemical properties of the protein was evaluated according to the change in the alkaline buffer capacity of aqueous solutions of the protein using hemoglobin as an example. Oxyhemoglobin solutions at a concentration of $5 \cdot 10^{-5}$ mol/L were used. Oxyhemoglobin solutions were made by osmotic hemolysis of red blood cells of white mongrel male rats weighing 180-240 g (n = 95). The amount of hemoglobin was monitored spectrophotometrically.

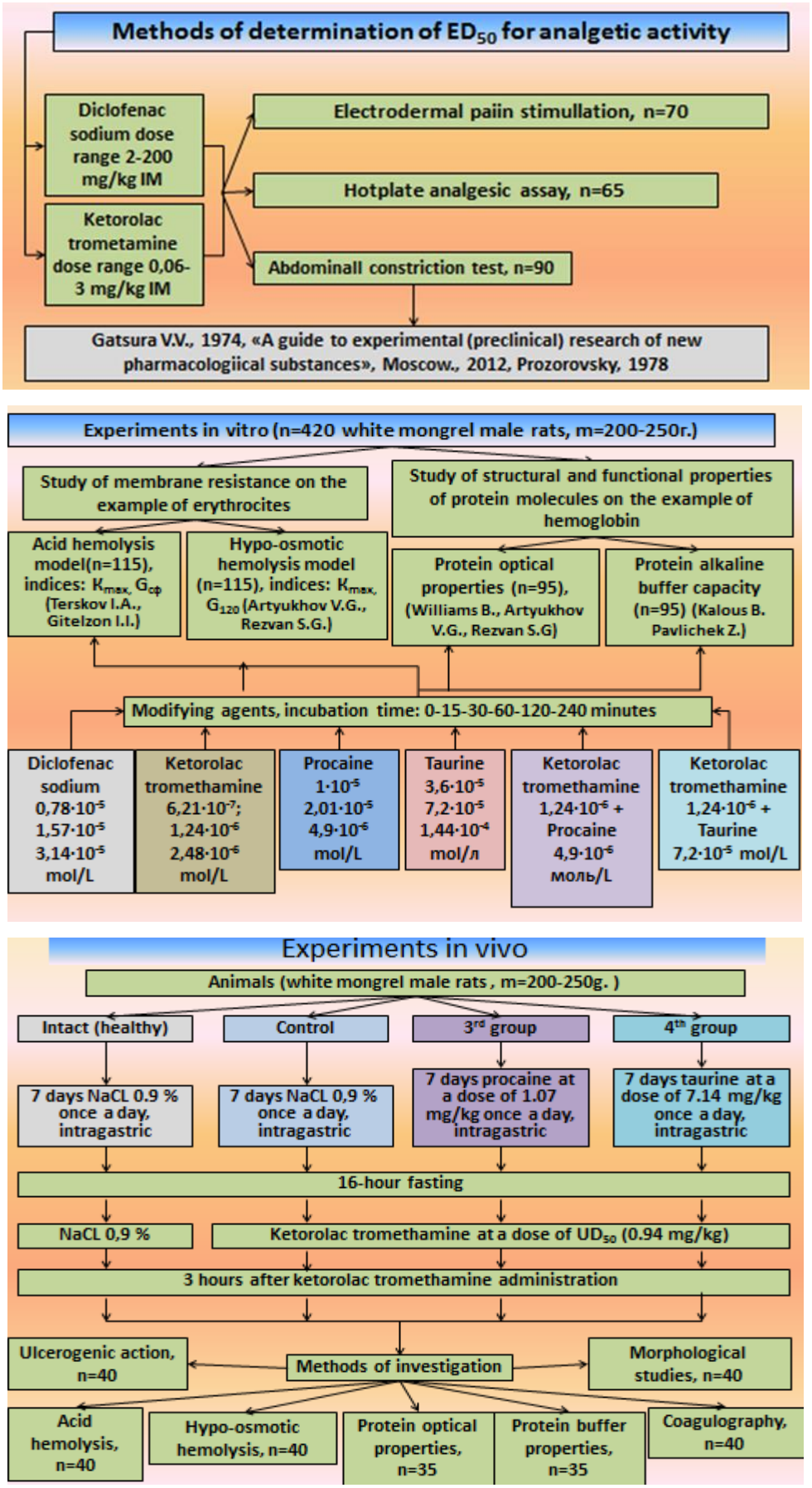

Fig. 1. The study design

The kinetics of the buffering capacity of hemoglobin was evaluated by means of "pH-150M" pH-metering device (RUE "Gomel Plant of Measuring Devices", Belarus), "TS-1/80SPU" electric dry-air thermostat, JSC "Smolenskoye SKTB SPU", Russia), "OPN-8" laboratory medical centrifuge ("TNK Dostan", OJSC, Kyrgyz Republic), "MS-01" magnetic stirrer, "ELMI" Ltd., Latvia), titrating a solution of intact or modified hemoglobin 0,1 M NaOH in the pH range 3.0-11.0 [21]. Substance concentrations in in vitro experiments, use pattern and dosing regimen in in vivo experiments, as well as the number of animal series correspond to the method of studying the structural and functional properties of erythrocyte membranes.

Pathomorphological studies

The number of series and animals in series, medical regimen, dosage regimen of procaine, taurine, ketorolac tromethamine are similar to the method used for studying ulcerogenic effects [16]. Internal organs (stomach, liver, kidneys) were taken to prepare histological preparations [22].

Reliability of the results obtained is based on the use of modern methods, instruments and devices, a sufficient number of experiments performed, representativeness of sample (728 specimens of laboratory animals, no less than 6 specimens per group) and adequate statistical processing of the information obtained using parametric criteria (Student's t-test) and nonparametric criteria (the Wilcoxon test and the Mann-Whitney U test).

Results

Study of the analgesic activity. The calculated effective dose (ED50) values required for further studies were 4.5 mg/kg for diclofenac sodium and 0.375 mg/kg for ketorolac tromethamine, according to abdominal constriction test.

Study of the efficacy of procaine and taurine for the prevention of ketorolac tromethamine ulcerogenic effect

It has been established that prophylactic intragastric administration of procaine at a dose of 1.07 mg/kg and taurine at a dose of 7.14 mg/kg for 7 days before administration of ketorolac tromethamine at an ulcerogenic dose UD50 (0.94 mg/kg) decreased the number of erosive ulcerative lesions of the stomach by 87.4% and 89.96%, respectively, compared to ketorolac tromethamine administered alone. Omeprazole provided no cases of ulcers in animals, but the long-term use of proton pump inhibitors (PPI) is characterized by a number of side effects and firstly by digestive disorders, which was confirmed by a decrease in feed intake and a consequent decrease in body weight by 10% in animals of this experimental group.

Pathomorphological studies

It has been established that the use of ketorolac tromethamine at a UD50 dose (0.94 mg/kg) after a 16-hour fasting was accompanied by multiple small necrotic foci in the apical part of the gastric glands, as well as foci of ulceration, destruction with deeper damage to the gastric mucosa and a decrease in the mucous layer density (increased amount of thinned areas). These changes may be induced by the main mechanism of ketorolac tromethamine action (blocking COX isoforms) and direct damage to the cell membranes of gastric mucosa.

Table 1

Study of the efficiency of procaine and taurine for the prevention of ketorolac tromethamine ulcerogenic effects (M±m)

Animal group	Number of animals in the group	Number of gastric mucosal ulcers, pcs.
Control	10	0
Ketorolac tromethamine 0.94 mg/kg	10	13.25±6.67*
Ketorolac tromethamine 0.94 mg/kg + Procaine 1.07 mg/kg	10	1.67±1.5*
Ketorolac tromethamine 0.94 mg/kg +Taurine 7.14 mg/kg	10	1.33±1.03*
Omeprazole 111.43 mg/kg	10	0

Note: * – the differences are statistically significant at $p<0.05$

After the prophylactic taurine administration to animals at a dose of 7.14 mg/kg or procaine (1.07 mg/kg) before ketorolac tromethamine administration at a dose of UD50, the gastric mucosa was better preserved and the defects were much less pronounced; there were insignificant isolated foci of desquamation of the apical part of the glands and dystrophy of the mucous membrane covering layer. Along with this, the gland mucous layer density compared to ketorolac tromethamine alone increased and approached the control value. Structural organization of the liver and kidneys in all groups remains within the norm.

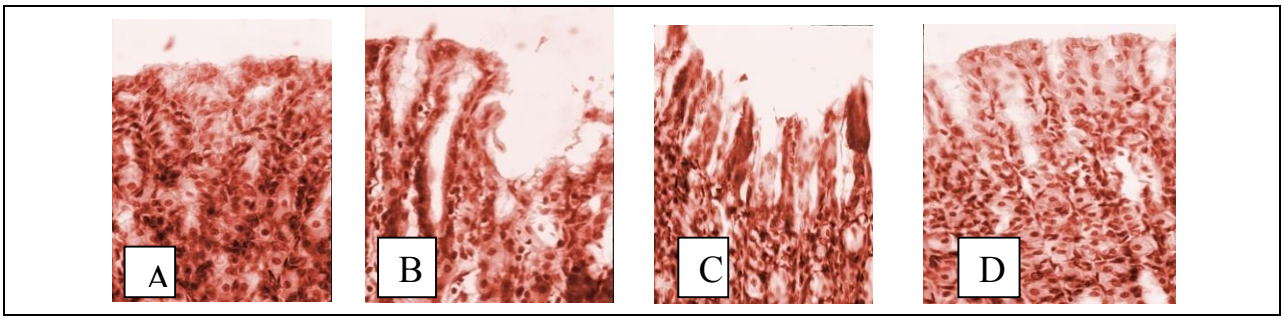

Fig. 2. Histoarchitecture of the gastric mucosa in the prevention of ketorolac tromethamine-induced NSAID-gastropathy by taurine and procaine
Note: A) control; B) ketorolac tromethamine; C) ketorolac tromethamine + taurine; D) ketorolac tromethamine + procaine (Color. gem-eosin. Magnification, appr. 7, v. 40)

Study of structural and functional properties of membranes for erythrocytes modified with sodium diclofenac and ketorolac tromethamine. Review of K_{max} for acid hemolysis (Table 2, 3), which characterizes the hemolytic sensitivity of mid-resistant erythrocytes, showed that sodium diclofenac at concentrations of $0.78 \cdot 10^{-5}$; $1.57 \cdot 10^{-5}$; $3.14 \cdot 10^{-5}$ mol/L and ketorolac tromethamine at concentrations of $6.21 \cdot 10^{-7}$; $1.24 \cdot 10^{-6}$; $2,48 \cdot 10^{-6}$ mol/L with incubation for 0, 15, 30, 60, 120 and 240 minutes in experiments *in vitro* results in dose-dependent increase (compared to the control) in the amount of erythrocytes concurrently entering the hemolysis stage. Thereafter they increase the hemolysis rate by 56.6% -1226.9% with the use of diclofenac sodium and by 27% -132% when introducing ketorolac tromethamine into the erythrocyte suspension, as a result of the formation of structural defects in cell membranes. Along with this, erythrocyte subpopulations have been conditionally classified under low-mid-high- and super-resistant subpopulations, which is represented by a change of modification processes for a destructive process, manifested in the presence of maximal and minimal values of K_{max}. Increase in the concentration of sodium diclofenac and ketorolac tromethamine shortens the period of modification and subsequent destruction of erythrocyte membranes. This action of diclofenac sodium and ketorolac tromethamine is obviously associated with chemical binding to protein-lipid complexes, which leads to the membrane damage and a decrease in the H + ion permeability threshold compared to the control value.

The proportion of spherocytes (G_{sph} index reflecting the structural and functional properties of a low-resistant population of erythrocytes) modified by diclofenac sodium at concentrations of $0.78 \cdot 10^{-5}$; $1.57 \cdot 10^{-5}$; $3.14 \cdot 10^{-5}$ mol/L and ketorolac tromethamine at concentrations of $6.21 \cdot 10^{-7}$; $1.24 \cdot 10^{-6}$; $2.48 \cdot 10^{-6}$ mol/L was lower than those in the control group by 100% -14.7% and 100% -17.6%, respectively, depending on the concentration and incubation time (see tables 2, 3). This indicates that the low-resistant population of erythrocytes also shows greater sensitivity to the action of these modifiers. However, in separate incubation regimens, the proportion of spherocytes exceeds the control value by 33% -140%, and in half the cases, when erythrocytes were modified by ketorolac trometamine, spherocytosis was not recorded. This points to a conditional division of low-resistant erythrocytes into subpopulations according to their resistance to acid hemolytic.

Table 2

Indices of acid and hypo-osmotic hemolysis of erythrocytes modified with sodium diclofenac depending on incubation time (M±m)

Concentrations, mol/L	K_{max} of acid hemolysis					
	Incubation time, min					
	0	15	30	60	120	240
Control	0.709±0.07	0.71±0.08	0.705±0.06	0.704±0.13	0.703±0.13	0.717±0.13
$0.78 \cdot 10^{-5}$	1.11±0.1*	1.88±0.07*	2.25±0.11*	3.27±0.13*	1.80±0.2*	1.483±0.25*
$1.57 \cdot 10^{-5}$	2.747±0.1*	3.08±0.08*	4.71±0.13*	4.35±0. 2*	3.73±0.13*	3.487±0.19*
$3.14 \cdot 10^{-5}$	3.732±0.1*	6.314±0. 1*	5.671±0.2*	5.15±0.25*	5.15±0.38*	9.514±0.5*
	G_{sph} (%) of acid hemolysis					
Control	4.0±0.32	3.4±0.2	3.6±0.22	3.8±0.2	3.9±0.25	3.6±0.45
$0.78 \cdot 10^{-5}$	7.6±0.35*	2.6±0.2*	2.3±0.19*	1.8±0.32*	5.2±0.26*	3.6±0.45
$1.57 \cdot 10^{-5}$	0*	0.3±0.06*	0.6±0.13*	1.9±0.32*	2.5±0.25*	0.8±0.13*
$3.14 \cdot 10^{-5}$	0*	2.9±0.13*	2.9±0.13*	3.1±0.1*	1.7±0.13*	2.6±0.19*
	K_{max} (relative units) of hypo-osmotic hemolysis					
Control	4.7±0.27	4.7±0.36	5.1±0.3	4.7±0.36	5.1±0.34	4.7±0.36
$0.78 \cdot 10^{-5}$	5.1±0.36*	7.1±0.4**	9.5±0.55**	8.1±0.45**	8.1±0.45**	8.1±0.45**
$1.57 \cdot 10^{-5}$	7.1±0.19**	9.5±1.0**	9.5±0.55**	8.2±0.4**	8.1±0.45**	8.1±0.45**
$3.14 \cdot 10^{-5}$	7.1±0.19**	14.3±1.2**	9.5±0.55**	14.3±1.25**	14.3±1.2**	19.1±1.7**
	G_{120} (%) of hypo-osmotic hemolysis					
Control	11.64±1.5	11.64±1.5	11.66±1.9	11.64±2.1	11.66±1.95	11.64±1.5
$0.78 \cdot 10^{-5}$	25.11±2.3**	28.64±2.5**	32.01±3.1**	33.0±3.1**	33.5±3.2**	33.7±3.5**
$1.57 \cdot 10^{-5}$	29.21±2.1**	33.13±3.1**	34.25±3.5**	34.42±3.5**	36.45±3.7**	40.9±3.8**
$3.14 \cdot 10^{-5}$	34.67±2.5**	35.48±2.5**	37.41±3.2**	41.05±3.0**	43.52±3.5**	48.9±4.0**

Note: ** – the differences are statistically significant at p <0.001, * – the differences are statistically significant at p <0.05

Table 3

Indices of acid and hypo-osmotic hemolysis of erythrocytes modified with ketorolac tromethamine depending on incubation time (M±m)

Concentrations, mol/L	Incubation time, min					
	0	15	30	60	120	240
	K_{max} of acid hemolysis, relative units					
Control	3.3±0.16	2.2±0.15	2.4±0.15	3.5±0.17	3.7±0.18	3.7±0.18
$6.21 \cdot 10^{-7}$	1.9±0.1**	5.1±0.2**	4.3±0.2**	6.3±0.3**	5.1±0.2**	5.8±0.3**
$1.24 \cdot 10^{-6}$	2.5±0.1**	4.7±0.2**	4.3±0.2**	7.1±0.3**	5.1±0.3**	6.3±0.3**
$2.48 \cdot 10^{-6}$	2.6±0.1**	5.1±0.2**	4.3±0.2**	5.8±0.3**	4.7±0.3**	2.9±0.2**
	G_{sph} of acid hemolysis, %					
Control	-1.5±0.1	-2.7±0.2	-3.4±0.2	-3.6±0.2	-2.7±0.14	-1.4±0.11
$6.21 \cdot 10^{-7}$	0**	0.6±0.03**	1.2±0.1**	0.3±0.01**	1.0±0.1**	1.7±0.1**
$1.24 \cdot 10^{-6}$	-2.0±0.1**	0.2±0.1**	-0.5±0.15**	0.8±0.1**	-0.6±0.03**	0.8±0.05**
$2.48 \cdot 10^{-6}$	-3.6±0.2**	-3.6±0.2**	-2.8±0.14**	-1.7±0.1**	-2.0±0.1**	-2.8±0.2**
	K_{max} of hypo-osmotic hemolysis, relative units					
Control	6.314±0.32	6.314±0.32	6.314±0.35	7.115±0.36	7.115±0.36	7.115±0.4
$6.21 \cdot 10^{-7}$	7.115±0.4**	11.43±0.6**	9.5±0.5**	7.115±0.41	11.43±0.5**	11.4±0.6**
$1.24 \cdot 10^{-6}$	9.514±0.5**	11.43±0.5**	9.514±0.5**	8.1±0.5**	9.5±0.48**	9.5±0.48**
$2.48 \cdot 10^{-6}$	11.43±0.5**	11.43±0.5**	9.514±0.5**	11.43±0.5**	9.514±0.4**	8.144±0.4**
	G_{120} (%) of hypo-osmotic hemolysis					
Control	24.2±2.1	26.0±1.4	27.0±1.3	29.0±1.51	29.0±2.0	29.0±1.7
$6.21 \cdot 10^{-7}$	24.4±1.5	29.8±1.5**	27.0±1.4	21.8±1.8**	22.8±1.1**	25.1±1.3**
$1.24 \cdot 10^{-6}$	29.7±1.5**	38.4±2.0**	34.6±1.8**	32.4±1.6*	27.0±1.4	31.0±1.6
$2.48 \cdot 10^{-6}$	39.4±2.5**	37.5±1.9**	37.3±1.9**	35.5±2.0**	31.4±1.8	31.3±1.8**

Note: ** – the differences are statistically significant at p <0.001, * – the differences are statistically significant at p <0.05

Analysis of the kinetics of erythrocyte hypo-osmotic hemolysis (in *in vitro* experiments) showed that addition of diclofenac sodium at concentrations of $0.78 \cdot 10^{-5}$; $1.57 \cdot 10^{-5}$; $3.14 \cdot 10^{-5}$ mol/L and ketorolac tromethamine at concentrations of $6.21 \cdot 10^{-7}$; $1.24 \cdot 10^{-6}$; $2.48 \cdot 10^{-6}$ mol/L to the incubation medium increases dose-dependently the number of latent defects of erythrocyte membranes, which is confirmed by an increase in the values of K_{max} for hypo-osmotic hemolysis by 8.5% -306.4% and 12.7% -81%, respectively, compared to the control (Tables 1, 2). This is confirmed by a dose-dependent increase in the proportion of hemolyzed erythrocytes by 115.7% -320.8% compared to the control using diclofenac sodium at the concentrations specified when observed for 120 seconds of the experiment (G_{120}) (Table 1). At concentrations of $1.24 \cdot 10^{-6}$; $2.48 \cdot 10^{-6}$ mol/L G_{120} values (Table 2) exceed the control level by 8% -62.8%, and at ketorolac tromethamine concentrations of $6.21 \cdot 10^{-7}$ mol/L they either exceed the control level by 14.6% or they are less than the control level up to 24.8%. This indicates the manifestation of modifying reactions for the more resistant red blood cells compared to the control. As incubation time and/or dose increase, modifying reactions are replaced by the prevalence of destructive processes. These changes probably indicate the interaction of sodium diclofenac and ketorolac tromethamine with spectrin-actin and lipo-stromatin complexes, i.e. the cytoskeleton of the membrane, which presents in a decrease in the erythrocyte hypo-osmotic resistance by means of membrane destruction and increased permeability for water and ions.

Study of the structural and functional properties of membranes for erythrocyte modified with procaine and taurine

The values of the main indices of acid and hypo-osmotic hemolysis erythrograms are presented in Tables 4-5. The amount (%) of erythrocytes modified with procaine at concentrations of $4.9 \cdot 10^{-6}$; $1 \cdot 10^{-5}$; $2.01 \cdot 10^{-5}$ mol/L, simultaneously entering the stage of proper acid hemolysis (K_{max}) in all cases is less than the control by 6.6% -38.5% or equal to it (Table 3). Analysis of spherocytosis for erythrocytes, modified with procaine at concentrations of $4.9 \cdot 10^{-6}$; $1 \cdot 10^{-5}$; $2.01 \cdot 10^{-5}$ mol/L with incubation time 0 to 240 minutes, showed that the spherocyte amount (G_{sph}) in the vast majority of cases is more than the control by 15-170%, but in a number of experiments it is equal to or less than the control (Table 3).

Table 4

Indices of acid and hypo-osmotic hemolysis for erythrocytes modified by procaine depending on incubation time (M±m)

Concentrations, mol/L	K_{max} of acid hemolysis, relative units					
	Incubation time, min.					
	0	15	30	60	120	240
Control	3.7±0.19	3.7±0.27	3.7±0.21	3.5±0.18	3.5±0.19	3.5±0.19
$2.01 \cdot 10^{-5}$	3.7±0.2	2.7±0.15**	2.7±0.18**	3.1±0.16*	2.9±0.16*	2.9±0.15*
$1.0 \cdot 10^{-5}$	3.5±0.18	2.7±0.14**	2.7±0.15**	2.6±0.2**	2.5±0.13**	2.5±0.15**
$4.9 \cdot 10^{-6}$	3.5±0.21**	2.7±0.2**	2.4±0.15**	2.1±0.1**	2.6±0.22**	2.6±0.15**
	G_{sph} of acid hemolysis, %					
Control	-1.9±0.12	-1.7±0.1	-1.1±0.11	-1.2±0.1	-1.0±0.09	-1.0±0.1
$2.01 \cdot 10^{-5}$	-2.6±0.2**	-1.3±0.13*	-2.0±0.1**	-2.2±0.14**	-4.0±0.17**	-4.0±0.2**
$1.0 \cdot 10^{-5}$	-2.2±0.12*	-0.8±0.1**	-1.4±0.1*	-2.4±0.12**	-3.0±0.15**	-3.1±0.2**
$4.9 \cdot 10^{-6}$	-1.8±0.1	-1.2±0.1**	-1.6±0.12**	-1.2±0.13	-2.50.13**	-2.7±0.14**
	G_{120} (%)of hypo-osmotic hemolysis					
Control	44.1±2.5	35.4±2.1	37.1±1.9	36.6±1.6	40.4±1.9	40.4±2.1
$2.01 \cdot 10^{-5}$	32.6±1.7**	40.7±2.0*	38.4±2.3	40.8±2.1*	39.3±2.0	39.1±2.0
$1.0 \cdot 10^{-5}$	37.4±2.5**	40.0±2.3*	38.6±1.8	35.9±1.8*	32.8±1.6**	32.5±1.6**
$4.9 \cdot 10^{-6}$	44.3±3.0	36.2±1.9	36.4±2.2	35.0±1.8	38.9±1.9	39.0±2.0

Note: ** – the differences are statistically significant at $p < 0.001$, * – the differences are statistically significant at $p < 0.05$

Table 5

Indices of acid and hypo-osmotic hemolysis for erythrocytes modified with taurine depending on incubation time (M±m)

Concentration, mol/L	Incubation time, min.					
	0	15	30	60	120	240
	K_{max} of acid hemolysis, relative units					
Control	3.732±0.21	3.732±0.19	3.732±0.18	3.732±0.18	3.732±0.2	3.732±0.18
$1.44 \cdot 10^{-4}$	3.732±0.18	3.732±0.2	3.732±0.21	3.732±0.18	2.904±0.14**	2.475±0.13**
$7.2 \cdot 10^{-5}$	3.078±0.17*	3.078±0.16*	2.605±0.14**	2.1±0.11**	2.05±0.13**	1.963±0.1**
$3.6 \cdot 10^{-5}$	3.078±0.16*	3.078±0.16*	2.246±0.11**	1.963±0.11**	2.904±0.14**	2.904±0.15**
	G_{sph} of acid hemolysis, %					
Control	-1.94±0.18	-1.70±0.17	-1.12±0.15	-1.17±0.16	-1.02±0.12	-1.02±0.12
$1.44 \cdot 10^{-4}$	-2.9±0.15**	-3.4±0.21**	-3.4±0.17**	-5.2±0.25**	-5.26±0.25**	-5.59±0.27**
$7.2 \cdot 10^{-5}$	-4.4±0.27**	-4.9±0.25**	-3.69±0.19**	-5.07±0.25**	-6.38±0.39**	-6.77±0.34**
$3.6 \cdot 10^{-5}$	-5.41±0.3**	-5.90±0.3**	-6.20±0.31**	-7.42±0.37**	-6.44±0.35**	-6.44±0.32**
	K_{max} of hypo-osmotic hemolysis, relative units					
Control	6.3±0.31	6.314±0.32	6.314±0.29	6.314±0.43	6.314±0.35	6.314±0.27
$1.44 \cdot 10^{-4}$	2.9±0.16**	3.5±0.17**	3.7±0.18**	1.4±0.1**	1.2±0.11**	3.1±0.15**
$7.2 \cdot 10^{-5}$	2.9±0.14**	2.1±0.15**	1.9±0.1**	2.1±0.11**	0.7±0.1**	1.3±0.1**
$3.6 \cdot 10^{-5}$	4.7±0.23**	4.0±0.24**	2.475±0.15**	1.7±0.13**	3.1±0.16**	3.7±0.15**
	G_{120} (%) of hypo-osmotic hemolysis					
Control	44.1±2.72	30.2±1.91	30.8±2.13	30.9±1.5	31.8±1.64	31.8±1.6
$1.44 \cdot 10^{-4}$	16.4±1.5**	12.8±1.53**	14.3±0.74**	7.2±1.11**	4.3±0.56**	5.2±0.53**
$7.2 \cdot 10^{-5}$	15.4±0.81**	14.0±0.77**	12.4±0.88**	9.4±0.54**	5.7±0.47**	2.7±0.14**
$3.6 \cdot 10^{-5}$	15.3±0.13**	11.4±0.57**	8.7±0.45**	4.3±0.22**	4.9±0.25**	4.7±0.27**

Note: ** – the differences are statistically significant at $p < 0.001$, * – the differences are statistically significant at $p < 0.05$

At the same time, more intensive development of the prehemolytic stage indicates an increased sensitivity of the "low-resistant" erythrocyte population to acid hemolytics. From this it follows that procaine increases the permeability of "low-resistant" erythrocytes membranes for H + ions, for "mid-" and "high-resistant" erythrocytes the use of procaine leads to modification, which is performed by increasing the barrier of permeability for H + ions.

When using taurine at concentrations of $3.6 \cdot 10^{-5}$; $7.2 \cdot 10^{-5}$; $1.44 \cdot 10^{-4}$ mol/L, prehemolytic phase is within 120 seconds, while for the control group it is in the range of 30-60 seconds, which indicates a certain delay in the development of the phase of RBC hemolysis proper. The number of erythrocytes simultaneously entering the proper hemolysis stage (K_{max}) did not exceed the control or was less than the control by17.5% -47.4% (Table 4). At the same time, the amount of spherocytes against the background of taurine administration exceeded the control by 48.5% -563.7% (Table 4). These changes indicate an increase in the acid resistance of erythrocytes, apparently due to the interaction of taurine with protein-lipid membrane complexes and its antioxidant, membrane-stabilizing action.

Addition of procaine at concentrations of $4.9 \cdot 10^{-6}$; $1 \cdot 10^{-5}$; $2.01 \cdot 10^{-5}$ mol/L to the suspension of erythrocytes, in conditions of hypo-osmotic environment, does not affect the rate of hypo-osmotic hemolysis, and taurine at concentrations of $3.6 \cdot 10^{-5}$; $7.2 \cdot 10^{-5}$; $1.44 \cdot 10^{-4}$ mol/L reduces it by 25.5% -88.1% (Table 3, 4). However, the structural and functional state of erythrocytes more resistant to the hypo-osmotic environment (G_{120}) is characterized by the dose-dependent interaction of procaine with the spectrin-actin and lipo-stromatin membrane complexes. And with an increase in the dose of procaine to $1.0 \cdot 10^{-5}$ mol/L; $2.01 \cdot 10^{-5}$ mol/L and the incubation time, possibly due to the formation of a larger number of complexes, some degradation of the

cytoskeleton of erythrocyte membranes is observed, that is, formation of latent membrane defects. At the procaine concentration of $4.9 \cdot 10^{-6}$ mol/L, such changes were not observed and the amount of hemolyzed erythrocytes within 120 seconds of the experiment did not exceed the control level (Table 3). In case of using taurine at the indicated concentrations, in all experiments, the G_{120} values were less than the control by 63% -91.5% (Table 4).

Study of structural and functional properties of membranes for erythrocytes modified with ketorolac tromethamine in combinations with procaine and taurine

In experiments *in vivo*, animals were prophylactically given intragastric procaine or taurine at doses of 1.07 mg/kg and 7.14 mg/kg for 7 days, and ketorolac tromethamine at a dose of UD50 was administered on day 8. The results are presented in Tables 6, 7.

Table 6

Indices of acid and hypo-osmotic hemolysis of erythrocytes modified with ketorolac tromethamine in combination with procaine and taurine (M±m)

Group	K_{max} of acid hemolysis, relative units Incubation time, min.					
	0	**15**	**30**	**60**	**120**	**240**
Control	4.011±0.2	4.011±0.23	4.011±0.21	3.732±0.19	3.732±0.22	3.732±0.18
Taurine + Ketorolac tromethamine	4.011±0.24	4.011±0.2	3.271±0.18**	4.331±0.23*	4.331±0.21*	4.331±0.25*
Procaine + Ketorolac tromethamine	5.2±0.25**	5.2±0.25**	5.145±0.28**	5.671±0.27**	5.671±0.29**	6.314±0.32**
	G_{sph} of acid hemolysis, %					
Control	-1.936±0.11	-1.698±0.11	-1.121±0.1	-1.166±0.12	-1.016±0.1	-1.016±0.07
Taurine + Ketorolac tromethamine	-3.7±0.19**	-4.5±0.22**	-4.6±0.23**	-5.1±0.21**	-5.7±0.25**	-5.4±0.27**
Procaine + Ketorolac tromethamine	-2.2±0.11	-1.3±0.1*	-1.5±0.14*	-2.9±0.16**	-2.56±0.13**	-4.35±0.18**
	K_{max} of hypo-osmotic hemolysis, relative units					
Control	5.145±0.25	4.705±0.23	4.705±0.24	4.705±0.23	4.705±0.23	4.705±0.23
Taurine + Ketorolac tromethamine	4.7±0.25	3.7±0.19**	3.7±0.2**	2.9±0.17**	3.7±0.21**	3.7±0.18**
Procaine + Ketorolac tromethamine	3.5±0.18**	4.7±0.24	5.1±0.26	5.1±0.25	5.7±0.29**	6.3±0.32**
	G_{120} (%)of hypo-osmotic hemolysis					
Control	22.2±1.51	22.9±1.15	21.8±1.14	23.6±1.25	23.4±1.17	22.7±1.14
Taurine + Ketorolac tromethamine	19.8±1.13	15.1±1.21**	13.0±1.43**	10.9±0.94**	9.2±1.11**	7.2±0.73**
Procaine + Ketorolac tromethamine	15.8±0.82**	11.8±1.32**	12.3±1.15**	11.4±0.87**	9.1±0.57**	9.1±0.57**

Note: ** – the differences are statistically significant at $p < 0.001$, * – the differences are statistically significant at $p < 0.05$

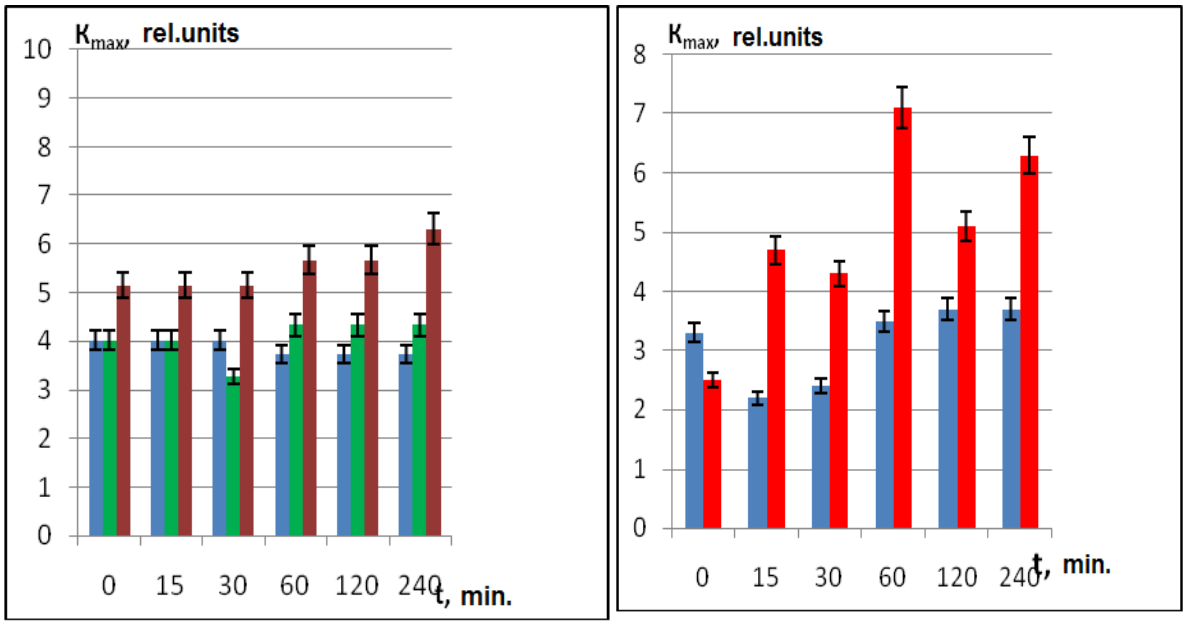

Fig. 3. The values of K_{max} (relative units) of erythrocyte acid hemolysis against the background of the use of ketorolac tromethamine and its combination with taurine and procaine.
Note: contro ; taurine + ketorolac tromethamine ; procaine + ketorolac tromethamine ; etorolac tromethamine

Analysis of the combined use (in *in vitro* experiments) of taurine (7.2 x 10^{-5} mol/L) and procaine (4.9 x 10^{-6} mol/L) with ketorolac tromethamine (1.24 x 10^{-6} mol/L) allow us to conclude that procaine and, to a greater extent, taurine, prevent ketorolac tromethamine-induced damage to erythrocyte membranes. This is confirmed by a significant decrease in the rate of acid hemolysis (up to 57%) for the main mid-resistant erythrocyte population, a significant increase in the amount (%) of erythrocytes that are simultaneously in the stage of spherulation (maximum by 100%), and the duration of the prehemolytic stage of hemolysis (maximum by100%) against the background of combined use of these modifiers compared to the use of ketorolac tromethamine alone.

Table 7

Values of K_{max}, G_{sph} and G_{120} indices of acid and hypo-osmotic hemolysis against the combination of ketorolac with procaine and taurine *in vivo* (M±m)

Name	Acid hemolysis		Hypo-osmotic hemolysis	
	K_{max}, relative units	G_{sph},%	K_{max}, relative units	G_{120},%
Control	4.3±0.25	-0.9±0.05	5.7±0.29	42.9±2.15
Ketorolac tromethamine	7.1±0.35**	-1.1±0.06*	19.1±0.75**	62.3±3.12**
Ketorolac tromethamine + Taurine	5.7±0.28**	-2.4±0.12**	4.3±0.27*	25.8±1.97**
Ketorolac tromethamine + Procaine	5.7±0.31**	-2.2±0.11**	5.7±0.36	30.6±2.57**

Note: ** – the differences are statistically significant at p <0.001, * – the differences are statistically significant at p<0.05

Analysis of the hypo-osmotic erythrogram faindings reveals that the introduction of procaine and taurine into the suspension of erythrocytes prevents the formation of latent defects induced by ketorolac tromethamine. This is reflected in a decrease in the rate of erythrocyte hypo-osmotic hemolysis when combined with taurine by 8.6% – 38.3% compared to the control. In case of combined use of ketorolac tromethamine with procaine, the hemolysis rate was less than or equal to the control during incubation up to 30 min, and it increased by 9.4% -34.2% in the period of 30-240 minutes compared to the control. When ketorolac tromethamine used alone, K_{max}

exceeded the control by 14.5% -81%. Besides, G_{120} values for hypo-osmotic hemolysis against the combined use of procaine and taurine with ketorolac tromethamine with an incubation time of 0-240 minutes decreased by 10.8% -68.1% compared to the control. While for the use of ketorolac tromethamine alone, the G_{120} values exceeded the control by 6.9% -47.7%.

Analysis of the results of the *in vivo* experiments (Table 6) showed that the preliminary intragastric administration of taurine (7.14 mg / kg) and procaine (1.07 mg / kg) for 7 days reduces the damaging effect of ketorolac tromethamine at a UD50 level (0.94 mg kg) for a population of mid-resistant red blood cells under conditions of acid hemolysis. This is confirmed by the fact that the acid resistance of the main erythrocyte population when using ketorolac tromethamine at a dose of UD50 is reduced by 64.3% relative to control, and with the preventive administration of taurine and procaine – by 30.9%. At the same time, against the background of combined use of taurine and procaine with ketorolac tromethamine, the proportion of erythrocytes which are simultaneously at the stage of spherulation is increased by 115.99% and 93.2% compared to the use of ketorolac tromethamine alone. Also in both cases of combined use, the erythrogram fold area is reduced which indicates that the final level of hemolysis has been reached for a longer period of time.

Comparing the data of hypo-osmotic erythrograms (*in vivo*), it can be concluded that the prophylactic use of taurine (7.14 mg / kg) and procaine (1.07 mg / kg) before administration of ketorolac tromethamine at a dose of UD_{50} (0.94 mg / kg) decreases the number of latent defects in erythrocyte membranes, as evidenced by a decrease in the rate of hypo-osmotic hemolysis with prophylactic administration of taurine and procaine by 77.3% -70.3% and G_{120} by 58.58% -50.9%, respectively, compared to the use of ketorolac tromethamine alone.

Study of the effect of diclofenac sodium, ketorolac tromethamine, procaine, taurine and their combinations on the optical properties of proteins

In experiments *in vitro*, diclofenac sodium was injected into the hemoglobin solution at concentrations of 0.78×10^{-5}; $1.57 \cdot 10^{-5}$; $3{,}14 \cdot 10^{-5}$ mol/L and ketorolac tromethamine ($6.21 \cdot 10^{-7}$, $1.24 \cdot 10^{-6}$, $2.48 \cdot 10^{-6}$ mol/L) against the background of a 30-minute incubation at 55 ° C promotes an increase in light absorbance, and hence the denaturation depth by 15.1%; 17.82%; 42.15% and 14.35%; 19.4%; 35.65% respectively, compared to the control. These changes are evidently caused by a decrease in the number or weakening of intramolecular bonds (hydrogen bonds) that stabilize the space structure of the protein, which manifest themselves as a dose-dependent increase in the denaturation level under conditions of thermal incubation compared to the control. The introduction of procaine into the solution of oxyhemoglobin at concentrations of $4.9 \cdot 10^{-6}$ mol/L; $1 \cdot 10^{-5}$ mol/L; $2.01 \cdot 10^{-5}$ mol/L was accompanied by changes in the light absorbance, which nature was opposite to the experiments with sodium diclofenac and ketorolac tromethamine, which was reflected in a decrease in the denaturation depth by 2.95%; 9.44% 17.6%, respectively, compared to the control. These changes may indicate some consolidation of the protein molecule under these conditions. A single application of taurine does not cause a change in the optical properties of this biopolymer under conditions of thermal incubation compared to the control. The combined use (in *in vitro* experiments) of procaine ($4.9x10^{-6}$ mol/L) and taurine (7.2x10-5 mol/L) with ketorolac tromethamine ($1.24x10^{-6}$ mol/L) demonstrated the denaturation depth to exceed the control by 7.43% -15.1%, respectively. When ketorolac was used at this dose alone, the light absorbance exceeded the control by 19.44%. This indicates a decrease in the denaturation depth for oxyhemoglobin under the influence of procaine in combination with ketorolac tromethamine compared to the use of the latter alone. The results of studying the optical properties of oxyhemoglobin, modified by ketorolac tromethamine and its combined use with procaine and taurine in *in vivo* experiments were fairy close to the control and showed no statistically reliable differences from the control, but a tendency to decrease the degree of denaturation against the background of preliminary use of procaine and taurine was observed.

Study of the effect of sodium diclofenac, ketorolac tromethamine, procaine, taurine and

their combinations on the buffer properties of proteins

In experiments *in vitro*, sodium diclofenac at concentrations of 0.78 • 10^{-5}; 1.57 • 10^{-5}; 3.14 • 10^{-5} mol/L was found to cause conformational changes in the oxyhemoglobin molecule, which are accompanied by an increase in the buffer capacity of this protein due to the dissociation of H +. When using ketorolac tromethamine at concentrations of 6.21 • 10^{-7}; 1.24 • 10^{-6}; 2.48 • 10^{-6} mol/L, a similar situation persisted, which was manifested by an increase in the alkaline buffer capacity mainly due to an increase in the dissociation of the NH^+ groups of the imidazole ring of histidine, terminal α-amino groups (by 10.5%, 13.13%; 17.2% compared to the control, respectively) and dissociation of sulfhydryl groups of cysteine, phenolic tyrosine groups, and ε-amino groups of lysine (by 8.7%, 11.76%, and 13.8% compared to the control, respectively). The use of procaine at concentrations of 4.9 • 10^{-6} mol/L; 1 • 10^{-5} mol/L; 2.01 • 10^{-5} mol/L was accompanied by a decrease in the buffer capacity of hemoglobin molecules mainly due to a decrease in the dissociation of the NH^+ groups of the imidazole ring of histidine, terminal α-amino groups (by 3.7%, 14.35%, 19.5%) and dissociation of sulfhydryl groups of cysteine, phenolic tyrosine groups, ε-amino groups of lysine (by 5.7%, 7.7%, 10.8%) compared to the control. Hemoglobin modification with taurine solution at concentrations of 3.6 • 10^{-5}; 7.2 • 10^{-5}; 1.44 • 10^{-4} mol/L was similar to the use of procaine (a decrease in the dissociation of NH^+ groups of the imidazole ring of histidine, terminal α-amino groups by 15.4%, 18.8%, 22.6% and sulfhydryl groups of cysteine , phenolic tyrosine groups, ε-amino groups of lysine by 1.9%, 4.2%, 5.51% compared to the control, respectively). These indices indicate conformational changes caused by the use of procaine and taurine at various concentrations, which are accompanied by protein molecule consolidation and / or a decrease in the number of ionic groups available for titration, possibly due to the procaine (taurine) binding to the corresponding functional groups, which leads to a decrease in the number of ionogenic groups determined in the appropriate ranges of pH.

In combination with *in vitro* (Table 8) of ketorolac tromethamine ($1.24x10^{-6}$ mol/L) with procaine (4.9x10-6 mol/L) and taurine ($7.2x10^{-5}$ mol/L), an increase in the alkaline buffer capacity was recorded by 5.95% and 8.3%, respectively, compared to the control, mainly due to an increase in the degree of dissociation of sulfhydryl groups of cysteine, phenolic tyrosine groups, and ε-amino groups of lysine, which is less than values of corresponding changes for ketorolac tromethamine alone. Therefore, it can be assumed that the combined use *in vitro* of ketorolac tromethamine with procaine and taurine to some extent prevents conformational changes in the hemoglobin molecule, which manifest themselves as molecule unfolding and increase in the number of ionic groups available for titration.

In experiments *in vivo* ketorolac tromethamine at a dose of UD_{50} was found to cause a change in the structural and functional properties of hemoglobin due to conformational transformation of the protein space structure, possibly due to molecule unfolding caused by breaking or weakening of bonds stabilizing it. This results in an increase in the buffer capacity, while procaine and taurine in these conditions *in vivo* do not affect the hemoglobin properties specified, however, there is a tendency to weaken the ketorolac tromethamine action on hemoglobin.

Table 8

Oxyhemoglobin buffer properties under the action of ketorolac tromethamine in combination with procaine and taurine (M±m)

Name, concentration, mol/L	pH /VNaOH intervals, μL		
	3 – 5	5 – 9	9 – 11
Control	422±8.37	101±8.22	605±19.40
Ketorolac tromethamine $1.24 \cdot 10^{-6}$ + Procaine $4.9 \cdot 10^{-6}$	424±11.40	104±8.22	641±23.02*
Ketorolac tromethamine $1.24 \cdot 10^{-6}$ + Taurine $7.2 \cdot 10^{-5}$	420±15.81	109±7.42	655±15.81*

Note: * – the differences are statistically significant at $p < 0.05$

Study of the blood coagulation system.

When studying the effect of ketorolac

tromethamine and its combined use with procaine and taurine on the coagulation system of blood, no significant changes were detected.

Discussion

Long-term administration of currently used marketed drugs that reduce the acidity of gastric juice (histamine H2- receptor blockers, proton pump inhibitors, antacids), increase intragastric pH and are capable of causing digestive disorders, which present in the clinical picture of dyspeptic syndrome. A prolonged increase in pH, on the one hand, significantly weakens the barrier to pathogenic and opportunistic flora entering the gastrointestinal tract. Persistent suppression of gastric secretion, on the other hand, causes hypergastrinemia, which is fraught with the development of dis- and metaplastic processes in the gastric epithelium (against the background of chronic inflammation) [12].

It is known that the enteral use of procaine in gastroduodenal ulcer at doses corresponding to the therapeutic range (0.25-0.5% solution up to 30-50 ml 2-3 times a day) is well tolerated and is characterized by the absence of side effects, in particular, due to the fact that it poorly penetrates through the mucous membranes [23]. In addition, procaine has a local anesthetic effect, providing a reduction in the severity of epigastric pain. It should be emphasized that procaine blocks the ion channels of the cell membrane and does not affect the acidity of the gastric juice.

Taurine is characterized by almost complete absence of side effects. Taurine has membrane-protective properties, normalizes the ratio of cell membrane phospholipids, regulates oxidative processes and exhibits antioxidant properties, reduces the degree of apoptosis for endothelial cells, prevents excessive calcium release from cells, has a number of cardiovascular benefits, has cardio-radio- and hepatoprotective properties, participates in the conjugation of bile acids and prevents cholestasis, participates in the regulation of GABA secretion, has hypoglycemic and hypolipidemic properties [24, 25].

Nevertheless, despite a number of properties that suggest the presence of a gastroprotective effect, procaine and taurine are not currently used in the clinical practice for the prevention of NSAID-induced damage to the gastric mucosa.

New components of the mechanism of the NSAIDs damaging effect on cell membrane and gastric mucosa have been revealed for the first time on the example of diclofenac sodium and ketorolac tromethamine in effective analgesic doses determined experimentally (diclofenac sodium 2.25 mg/kg – 9 mg/kg, ketorolac tromethamine 0.1875 mg/kg 0.75 mg/kg). These components involves changed structural and functional properties of protein molecules, which leads to a decrease in acid and hypo-osmotic resistance of cells. It has been found that procaine and taurine exhibit a membrane-protective effect, since they increase the acid and hypo-osmotic cell resistance to the action of damaging factors in *in vitro* and *in vivo* experiments. It is observed both in their use alone and in their combination with ketorolac tromethamine.

Conclusion

The mechanism of the diclofenac sodium and ketorolac tromethamine damaging effect on the gastric mucosa has been clarified, and a direct damaging effect on cell membranes has been revealed. It has been established that the use of procaine and taurine increases the resistance of cell membranes in conditions of acidic and hypo-osmotic environment, and also reduces damage to cell membranes caused by ketorolac tromethamine. The findings of the *in vitro* experiments are confirmed by *in vivo* experiments, demonstrating ability of procaine and taurine in their preventive administration to reduce the number of experimental erosive ulcers and changes of the gastric mucosal morphological structure induced by ketorolac tromethamine use. Obviously, the revealed efficiency of procaine in treating NSAID-gastropathy is associated with the ability to interact with membrane proteins and protein-lipid membrane complexes, causing changes in their conformation, and thereby changing the structural and functional properties of cell membranes, which leads to an increase in the permeability threshold for H +. The ability of taurine to inhibit ketorolac tromethamine-induced ulceration is possibly associated with its proven antioxidant and membrane-stabilizing properties, as well as the ability to normalize the ratio of phospholipids to cell membranes and the ratio of cholesterol to phospholipids. It follows that the prophylactic use of taurine (7.14 mg/kg) and procaine (1.07 mg/kg) before administration of ketorolac tromethamine (0.94 mg/kg) leads to an increase in gastric mucosal resistance. On this

evidence, original methods of protecting the gastric mucosa from the damaging effect of NSAIDs are proposed and consist of 7 days of prophylactic taurine (7.14 mg/kg) or procaine (1.07 mg/kg) dose. [26, 27, 28].

Besides, it was found that, both for the administration of ketorolac tromethamine alone and for its combined use with procaine or taurine, there were no changes in the structural organization of the liver and kidneys, the coagulogram findings did not differ from the control, indicating no hepatotoxic, nephrotoxic and hematotoxic effects.

Conclusions

1. New components of the mechanism of the damaging effect of sodium diclofenac and ketorolac tromethamine on cell membranes have been revealed; they influence the ability to induce weakening or breaking of intramolecular bonds stabilizing the protein molecule, which is associated with the dissociation of NH^+ groups of the imidazole ring of histidine, terminal α-amino groups (not less than by 10.5% compared to the control), sulfhydryl cysteine groups, tyrosine phenolic groups, and ε-amino groups of lysine (by at least 8.7%), and presents in an increase in the denaturation depth.

2. Procaine and taurine *in vitro* increase the resistance of cells to the effects of damaging factors. This is proved by an increase in: a) acid resistance of red blood cells with the addition of procaine by no more than 38.5%; b) acid and hypo-osmotic resistance of erythrocytes with taurine use by no more than 47% and 88%, respectively.

3. The experiments *in vitro* and *in vivo* have shown that procaine and taurine reduce the damage to cell membranes caused by ketorolac tromethamine at a concentration equivalent to ED_{50} and a dose equal to UD_{50}: a) procaine at a concentration equivalent to a minimum therapeutic dose of 1.07 mg / kg – by 28% and 19.7%, respectively, and reduces the formation of latent membrane defects by no less than 69%; b) taurine at a concentration equivalent to an average therapeutic dose of 7.14 mg / kg – by 54% and 19.7%, respectively, and reduces the formation of latent defects by at least 74%; c) oral prophylactic procaine administration to rats at a dose of 1.07 mg / kg and taurine at a dose of 7.14 mg / kg reduces the number of erosive and ulcerative defects caused by ketorolac tromethamine at a dose equal to UD_{50} by 87% and 90%, respectively, and also prevents changes in the gastric mucosal morphological structure according to the histological studies.

4. Oral prophylactic procaine (at a dose of 1.07 mg / kg) and taurine (7.14 mg / kg) administration before the damaging action of ketorolac tromethamine (0.94 mg / kg dose) is associated with no changes in the liver and kidney histoarchitecture, which is indicative of no hepato- and nephrotoxic effects.

Conflicts of interest

The authors have no conflict of interest to declare.

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